SCIENTIFIC STATUS SUMMARY

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MICROBIAL MODELING

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Innovative foods that are not shelf stable are being manufactured in increasing numbers. Many of these foods rely primarily upon maintenance of proper refrigeration to prevent spoilage and ensure microbial safety. Traditionally, the risk of growth of pathogenic microorganisms and/or toxin production in foods was determined through the use of inoculated pack studies. In these studies, foods were inoculated with a specific amount of a pathogen and the growth or decline of the microorganism was monitored during storage by repeated sampling. Now, however, there are too many products, alternate ingredients, and process variations to conduct a complete laboratory evaluation of each possible contingency and potential foodborne pathogen for each product. Research using either microbial broth media or inoculated packs does not permit quantitative interpolation to untested conditions, particularly when two or more factors interact. For example, a study may evaluate storage treatments of 15°, 25°, and 37°C. Simply plotting the microbial growth at each storage temperature or estimating the microbial growth rates provides only a subjective estimate of what to expect at 30°C. The increasing power and widespread availability of personal computers now make it worthwhile to develop the extensive data bases and equations necessary to create predictive microbial models.

In essence, microbial modeling is the use of mathematical expressions to describe microbial behavior. This includes expressions that depict how bacterial populations change with time and how the rate of change is influenced by environmental conditions. The modeling of microbial populations—particularly those of foodborne pathogens, coined "predictive food microbiology"—has become an active field of research. Modeling, however, has always been an integral part of food microbiology. Most food scientists, for example, do not give a second thought to the fact that they are employing mathematical modeling when using D-values and z-values to describe the thermal resistance of bacteria.

Current microbial models can provide "first estimates" of microbial growth or survival/inactivation, improve laboratory efficiency by suggesting critical areas for experimental trials and inoculated pack studies, and facilitate development of hazard analysis critical control point (HACCP) programs. This summary briefly describes the most frequently used microbial models, their use, and their limitations. Reviews have been published on early modeling (Farber, 1986), the historical development of models (Whiting, 1994), modeling of shelf life and safety of controlled and modified atmosphere packaged foods (CAP/MAP) integrated with temperature changes (Labuza et al., 1992), modeling of *Clostridium botulinum* (Baker and Genigeorgis, 1993), modeling of growth (McMeekin et al., 1993), and the development of models and software for pathogen growth (Buchanan, 1993, 1992, 1991).

Types of Microbial Models

Models can be classified by the microbiological event studied, the modeling approach used, or the variables considered. Whiting and Buchanan (1993) recently proposed a three-level classification method described as primary, secondary, and tertiary. *Primary* level models describe how microbial numbers change with time in a specified environment. These include growth and inactivation/survival models.

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Indirect measures of microbial numbers (turbidity,electrical conductance) or metabolic products (toxins, substrates) also can be modeled. A D-value, the time for a population to decrease 90% at a constant temperature, is a primary model, as is a measure of a microorganism's growth rate during the exponential growth phase. *Secondary* level models indicate how parameters of primary models change with respect to one or more environmental or cultural factors (e.g., atmosphere, pH, temperature, and salt level). The z-value, the change in temperature needed to decrease the D-value by 90%, used in thermal processing is an example of a secondary model. *Tertiary* level modeling applications assist in the use of primary and secondary level models by identifying and using pertinent information to calculate and display desired graphs, predictions, and comparisons. Examples are mathematical expressions, software packages, and expert systems. At this level, the user need not be aware of the equations in the underlying primary and secondary level models.

Modeling Growth. Mathematical models for describing microbial growth range from relatively simple estimates based on growth/no growth responses to more complex treatments where each phase of the growth cycle is described quantitatively. An example of the former was developed by Meyer et al. (1989) to determine the effects of pH and water activity on growth of spoilage yeasts in different groups of foods.

The next level of complexity is the development of *time-to-growth* or *lag-time models* that are often used for studying *C. botulinum*. Here it is more important to estimate the time for spore germination/growth or toxin formation than it is to determine the growth rate of the bacterium. Secondary level regression equations are then developed to relate how environmental factors affect this time. This growth model has been used to determine the effects of sodium lactate and sodium chloride on the time within which toxin is formed by *C. botulinum* in temperature-abused turkey products (Maas, 1993), the effect of temperature and atmosphere on *C. botulinum* growth in fresh fish (Baker and Genigeorgis, 1990), and the interaction of seven environmental factors on detection of visible mold growth on bakery products (Smith et al., 1988).

Alternatively, lag times have been studied using Most Probable Number (MPN) techniques to predict the probability that a single *C. botulinum* spore will germinate and grow to produce toxin within a specific time (Lindroth and Genigeorgis, 1986). In this approach, a set of serial dilutions of a sample were observed frequently, and the MPN was determined at each dilution by observing the pattern of positive test tubes. Changes in the probability of spore growth during storage were then described by a combination of logistic and regression equations (Genigeorgis et al. 1991; Roberts et al., 1981).

Developing a primary model to describe growth kinetics (e.g., lag phase duration, specific growth rate, and maximum population density) of a microorganism can be as simple as using a ruler to measure the linear portion of the growth curve when the data are plotted on semi-log graph paper; however, during the past several years, use of sophisticated mathematical approaches has increased. The most widely used primary model for describing microbial growth has become the Gompertz equation (Gibson et al., 1987; Gibson and Roberts, 1986). This four-parameter, asymmetrical, sigmoidal equation is:

$$N = A + C \exp(-\exp(-B(t-M))) \quad (1)$$

where N is the $\log_{10}$ of colony forming units/mL, A is the $\log_{10}$ of the initial number (i.e., inoculum) of the microorganism, C is the $\log_{10}$ of the difference between initial and final numbers of microorganisms at the stationary phase, B is a slope term indicating the rate of growth at the inflection point, M is the time of the inflection point, and t is time. Indirect parameters more familiar to microbiologists can be calculated by:

$$\text{Growth rate (N/hr)} = BC/e \quad (2)$$

$$\text{Lag time (hr)} = M - (1/B) \quad (3)$$

$$\text{Generation time (hr)} = e \log(2)/BC \quad (4)$$

$$\text{Maximum population density (N)} = A + C \quad (5)$$

The modeler fits the equation to the data points and obtains values for the parameters (C, M, B) that are specific for that data. The Gompertz equation was selected because it fit microbial data better than other sigmoidal equations (Zwietering et al., 1990). Recently, Baranyi et al. (1993), Jones and Walker (1993), and Whiting and Cygnarowicz-Provost (1992) proposed mechanistic-based growth models to replace the empirical Gompertz model.

Secondary models that describe changes in parameters of the primary models (e.g., Gompertz A, C, B, and M terms) when the microorganism's environment is altered have generally been of three types: a response surface equation (multiple polynomial regression equations), the Arrhenius relationship, and the

square root (Bélehrádek) model. Response surface techniques were developed originally for process optimization; typically, the equations contain quadratic or cubic terms and their interactions. The logarithm of a parameter is frequently modeled as a means of making the variance homogeneous. Equations of this type are descriptive, fitting data containing several factors without any assumptions about the relationship between a factor and microbial behavior. The Arrhenius relationship is the logarithm of the rate constant versus the reciprocal of the temperature in K. Additive versions include terms for pH and a_w (Davey, 1989, 1991), for example:

$$\ln (k) = C_o + C_1/T + C_2/T^2 + C_3 a_w + C_4 a_w^2 \quad (6)$$

where k is the growth rate, T is the temperature, and C_0–C_4 are parameter values. The square root model is based upon the linear relationship between the square root of the rate and temperature (Ratkowsky et al., 1991):

$$\sqrt{k} = b(T - T_{min}) \quad (7)$$

where b is a slope parameter and T_{min} the temperature where the growth rate extrapolates to zero. This model has also been expanded to include the temperature of maximum growth rate, pH, and a_w (McMeekin et al., 1992):

$$\sqrt{k} = b[(a_w - a_{wmin})(pH - pH_{min})]^{0.5} (T - T_{min}) \quad (8)$$

where k is the measured rate, a_{wmin}, pH_{min}, and T_{min} are the respective parameter values extrapolated to a rate of zero for the factors a_w, pH, and T.

To date, the development of tertiary level programs for the growth of foodborne microorganisms has been limited. The Microbial Food Safety Research Unit of the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) Eastern Regional Research Center (ERRC) has released a program. This version provides a user-friendly format for application of available response surface models for broth cultures of *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Shigella flexneri*, *Bacillus cereus*, *Aeromonas hydrophila* and *Escherichia coli* O157:H7 (Buchanan, 1993). This DOS-based PC program has a series of menu screens for entering information on the desired microorganism, atmosphere, initial bacterial populations, pH, NaCl level, temperature, and $NaNO_2$ concentration. The program then provides estimates of growth kinetics values, time to reach a specified population, or a graph showing the expected growth curve. The variables can be modified easily, allowing the user to perform a rapid series of "what if" estimates. In the United Kingdom, the "Food Micromodel" program, developed by the Ministry of Agriculture, Fisheries, and Food, has predictive equations for growth of *Listeria*, *Yersinia*, non-proteolytic *C. botulinum*, *Aeromonas*, *Salmonella*, and *Staphylococcus*. Factors include temperature, pH, and a_w. Validation of these equations using foods from six major groups (meat, fish, vegetables, dairy products, bakery products, and eggs) is underway. To obtain predictions from this program, information on the food's composition and other relevant factors is provided to the program's microbiologist who runs the model and provides results and interpretations.

The next phase of the tertiary level is an expert system, several of which are under development. These are computer programs that emulate the reasoning and decision making of human experts. The programs contain descriptive information, equations, and logical rules. The user starts with a query, and the system applies rules to request additional information. Through dialog, the user retrieves or has desired information calculated. Descriptions of these systems have been given by Adair and Briggs (1993), Jones (1993), Voyer and McKeller (1993), and Zwietering et al. (1992).

Modeling Inactivation/Survival. Mathematical modeling of the behavior of foodborne microorganisms began in the 1920s with thermal death time calculations. D- and z-values have been used successfully by the canning industry for over half a century to avoid the hazard from botulinal toxin. An excellent anthology of the pioneering papers in the development of this model was edited by Goldblith et al. (1961). This primary model describes a simple first-order decrease in log numbers with heating time at a constant temperature. This model remains in use for many thermal inactivation studies (Fujikawa et al., 1992; Mohr and Simon, 1992; Mackey et al., 1990). Non-linear declines in the log number of survivors over time, however, are frequently observed and several alternate models have been developed to account for this behavior. For example, a population dynamics theory has been proposed to account for initial decreases or increases in spore populations. This theory includes a combination of first-order processes for the rapid inactivation of less heat-resistant spores followed by a period of activation of remaining spores to a more heat sensitive state, and finally, inactivation of remaining spores (Rodriquez et al., 1992, 1988; Teixeira and

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Rodriquez, 1990). These concepts were used to model inactivation of dormant spores in ultra-high temperature sterilization (Sapru et al., 1992). Bhaduri et al. (1991) inverted the sigmoidal Gompertz equation to describe the low-temperature thermal inactivation of *L. monocytogenes* that showed curves reflecting both an initial period of survival and a heat-resistant subpopulation.

Despite extensive use of models for thermal inactivation of foodborne microorganisms, there has been relatively little work done on the development of mathematical expressions to describe inactivation under ambient or chilled storage. When placed in an adverse environment, such as a low pH, microbial populations decline over time. Their survivor curves, however, frequently exhibit shoulders and tailing. The microbial inactivation rate often depends on combinations of inhibitory factors, none of which are adequate to cause death alone.

Several primary models have been used to depict the inactivation of foodborne pathogens during storage. Buchanan et al. (1993a) used a linear model incorporating a term for a lag phase duration followed by a first-order decrease to model the inactivation of *L. monocytogenes*. This model was used to study the interaction between pH and concentration of lactic or acetic acids. They observed that the logarithm of the time for 4 logs of inactivation was inversely related to the square root of the concentration of undissociated acid. A logistic model originally developed by Kamau et al. (1990) to describe the enhanced thermal destruction of *L. monocytogenes* and *S. aureus* by the lactoperoxidase system was modified to provide a primary model for the survival of foodborne pathogens (Whiting, 1993). This model allows depiction of both a shoulder and two slopes; the second slope is used to quantify tailing populations. Both the logistic and linear models are being used to quantify the effects of pH, NaCl levels, temperature, oxygen availability, and $NaNO_2$ concentration in microbiological media on the survival of *Salmonella* and *S. aureus* (Whiting, 1993) and *L. monocytogenes* (Buchanan et al., 1993a). These data were evaluated by response surface analysis to develop secondary models for estimating the survival of these pathogens in uncooked meat products and other foods.

Modeling Changing Conditions. Data for most models are acquired under constant conditions. Factors such as temperature, pH, water activity, or atmospheric composition, however, seldom remain constant during the manufacture and storage of a chilled food (Labuza et al., 1992). Under cycling conditions, a constant temperature can be determined, one that would result in the same growth under variable conditions. Frequently growth or inactivation is estimated for a short period of time for the average conditions of that period. The final population is estimated by the sequential calculation from one period to the next. This approach was used successfully to estimate bacterial growth during cooling of cooked meats (Gill and Jones, 1991; Blankenship et al., 1988; Gill, 1986). This approach, however, may be too simple in other circumstances. Fu et al. (1991) proposed a model for sinusoidally fluctuating temperatures, but found that abrupt transitions were not effectively modeled, i.e., a history effect exists. Abrupt transitions can lead to adjustment periods, the extent of which is dependent on the degree of change a microorganism experiences when shifted from the source medium to the test medium (Baranyi et al., 1993; Shaw, 1967). Additional research is needed to determine the best way to model fluctuating temperatures, changing pH values, varying a_w, or other food parameters that change during storage. Packaging material, thermal properties of the food, and package size affect the temperature of a food and would need to be incorporated into models.

APPLICATIONS

Microbial models are valuable tools for predicting the growth or survival of microorganisms. They are rapidly evolving from a subject of research and development into techniques used by the food industry and regulatory agencies and in general microbial research. Models are a means of rapidly obtaining an initial insight into a microorganism's behavior and are guides for evaluating potential problems. The USDA Food Safety and Inspection Service, for example, has used models to make assessments of pathogen growth in proposed meat product formulations. Models, however, do not completely replace microbial testing or the judgement of a trained and experienced microbiologist. They instead allow microbiological assessment to be conducted more rapidly, objectively, and cost effectively. Models can provide useful information for making decisions in many situations. These situations are described below.

Prediction of Safety and Shelf Life. Growth and survival models can estimate potential risks from pathogens in a food after a normal or expected abusive storage period. Growth models can aid in setting a "pull date" by estimating the time for attaining a specified population of spoilage or pathogenic microorganisms. Additionally, estimating microbial

behavior for a range of potential environmental factors for a new food can quickly highlight areas of concern and guide design of challenge tests, storage trials, or other techniques that will more precisely determine risk.

Quality Control. Models can aid in the development of HACCP programs by showing what conditions permit growth or survival and thereby identify critical control points. Quantitative estimates of microbial growth at various levels of environmental or compositional factors can indicate allowable ranges for that factor. This becomes particularly important in foods that rely on the interaction of several factors to control microbial growth. An out-of-process event, such as a food inadvertently lacking the intended salt or experiencing a period of inadequate refrigeration, can be evaluated for the microbial consequences. This assists in making more objective, consistent decisions to rework, rapidly utilize, or scrap a food or ingredient without waiting for testing.

Product Development. Changes in a food's composition or a new formulation can quickly be evaluated for pathogen growth or survival. Models show which factors have major influences on microbial populations and can compare new versus old formulations.

Education. Explanations for nontechnical people can be enhanced by models. By generating graphs or estimates of the time to a critical microbial population, models can demonstrate dramatically the importance of maintaining proper temperatures or the benefits that high-quality raw materials with low initial microbiological counts have on a food's microbiological safety and quality. The models are used also in food microbiology courses to illustrate the effects of factors that control microbial growth.

Laboratory Planning and Data Analysis. Efficiency is promoted when a model's predictions guide the design of a testing program. This saves resources, time, and money and permits the laboratory to concentrate its efforts on critical steps. Models are used routinely in the USDA/ARS/ERRC's Microbial Food Safety Research Unit laboratory to devise work schedules for sampling timed experiments.

Modeling is becoming a routine technique for analysis of microbial data. The mathematical fit provides an unbiased estimate of the parameter values that describe a particular microbial response. These values can then be averaged or otherwise manipulated mathematically and error estimates can be determined. Simply plotting a growth curve will no longer be an adequate presentation or analysis of microbial data.

Using mathematical models for the various tasks described above increases understanding of the factors that govern microbial growth or inactivation in foods, thereby providing processors greater confidence in the safety of their processes and products. In turn, this knowledge enables the manufacturer to create more sophisticated and effective HACCP programs.

Considerations

Most modeling uses a mixture of strains of microorganisms, since the growth or survival predicted by the model reflects the fastest growing or hardiest strain in the mixture, respectively. Ideally, mixtures of strains represent the most frequently occurring strains with "normal" characteristics that are likely to be present in foods. An atypical strain which is infrequently present, the heat-resistant *Salmonella senftenberg*, for example, should have a separate heat inactivation model. The model user would check both models and subsequently make an appropriate judgement.

Recent studies have indicated that the conditions under which inocula were grown can have a substantial impact on growth or inactivation kinetics. The data used to generate most models are based on cells grown to the late log or stationary phase in a favorable environment prior to being used to inoculate a test culture. Such cells can have differences in their lag times or survival characteristics from exponential growth phase cells, cells grown at nonoptimal temperatures, or cells pre-adapted to acidic or alkaline pHs. Models have generally been created using stationary-phase cells grown in glucose-containing media that produced a depressed pH. These cells tend to have longer lag phases and survival when placed in acid environments than cells maintained at neutral pH values.

Most microbial models are based on regression equations and are subject to the assumptions and cautions inherent with these types of analyses. A model should include all the factors that have a statistically significant effect on microbial growth or survival. Nearly all models are based upon laboratory studies using controlled temperature, pH, and NaCl level in a nutritionally rich medium. These three factors are often the most important factors governing microbial behavior in a food, but any important additional factor would have an impact on the accuracy of the model. In the three factor model (temperature, pH, and NaCl), for example, the presence of an unmodeled antimicrobial agent would typically result in

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over-estimated growth rates or survival times. Predictions from all models are most accurate in the central area of their design, i.e., where all experimental factors are close to their midpoint. As any factor changes, moving toward its limits, there will be greater variation in the predictions. Thus, extrapolating beyond the experimental ranges is not recommended.

An estimate of the variation about any prediction is desirable; most models to date, however, do not explicitly provide this information. The user needs to be aware of various error terms and any transformations used, e.g., modeling the log of the time parameter. As time increases, the confidence interval around a prediction increases. Often, estimates of the variability are relatively large due to the inherent variability of microbial systems, particularly those under stress conditions. One should remember, however, that while this is less than desirable, it is generally a much more objective way to estimate microbiological behavior than the "informed guesses" routinely made in the absence of models or other systematic means of evaluating microbiological data.

An integral part of model development must be validation of the models. General models for growth or survival are derived typically in broth media. After collecting an appropriate number of curves and calculating the primary and secondary models, it is important to test new trials against the regression equations and compare the closeness of fit. This provides an estimate of the "goodness of fit" and shows where additional data are needed. The next stage of validation is to test the predictions against microbial behavior in various foods. This will demonstrate the limitations of the model and, when predictions are poor, suggest additional factors necessary to make the model more widely applicable. Models cannot be used with confidence until this validation is done. Users of a model should conduct a sufficient number of trials with their products to ensure the validity of a model before accepting the model's predictions.

Many broth- and food-based models are designed to be "fail-safe." This means that the growth rate predicted from the model will be faster or a predicted lag time will be shorter than that which actually occurs in the food. The user should check the literature to determine whether this is the case.

Significant strides have been made in the past five years in the development of effective mathematical models for assessing the effects and interactions of several important variables (i.e., temperature, pH, water activity, and $NaNO_2$) on the growth of foodborne pathogens. The next level of sophistication, however, needs to be addressed. Currently, most models do not separate the effect of type and concentration of organic acid from the pH. The activities of commonly used antimicrobials, such as phosphates, sorbates, and bacteriocins, have not been quantified systematically. The use of humectants other than NaCl has not been evaluated adequately, nor have the impacts of microbial competition or time of spoilage been incorporated. Appropriate methods to model changing conditions, particularly the lag phase, are needed. These questions are being actively addressed by groups of investigators worldwide and continuing improvements in models are anticipated.

Because of the large data bases necessary for development of microbial models, international cooperation between modelers is increasing to expand the scope of the models and to avoid duplication of effort. The European Economic Community's "FLAIR" (Food Linked Agro Industrial Research) program promotes personnel exchanges, coordinated research, and data exchange. A major step forward in consolidating the international efforts in modeling was made in 1992 when the USDA/ARS organized the International Workshop on the Application of Predictive Microbiology and Computer Modeling Techniques to the Food Industry. The workshop was attended by scientists from seventeen countries (Buchanan et al., 1993b). The details of a four-country collaborative demonstration project are being finalized between the Australia, Canada, United Kingdom and United States, to share data bases and validate models for the growth of *L. monocytogenes* and other foodborne pathogens.

Ultimately, mathematical models will serve as an integral part of microbiological risk assessment by providing a means of calculating realistic exposure estimates. Three areas need to be quantitatively estimated to achieve an effective microbiological risk assessment (Albanese, 1992):

(1) identify sources of contamination, enumerate frequency of occurrence, and quantitate microbial numbers in raw ingredients;

(2) understand how pathogens will survive, grow, and/or produce toxins or other virulence-related factors under the conditions that exist in foods when they are present; and

(3) identify the human response to the pathogen and the infectious dose for various groups of people.

Methods to estimate the risk from low numbers of microorganisms were compared by Haas (1983) who concluded that it is impossible to rule out the hypothesis that a single microorganism has a probability of causing disease.

These three points must be integrated into an effective overall model that can be used to objectively set priorities for addressing microbiological food safety concerns. Current efforts in microbial modeling have made great strides in fulfilling the second area, but desired integration of risk assessment await advancements in the other two areas.

Conclusion

The progress in microbial modeling has been impressive. Models are now a standard research technique and are a powerful tool in designing foods and controlling food processes. While models provide increasingly sophisticated estimates of expected microbial behavior and a way for food scientists to work smarter and faster, they are not substitutes, however, for experienced judgement or well-designed laboratory testing.

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